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EVALUATION OF THE RELIABILITY OF THE MOUSE EPIDIDYMAL SPERM ANEUPLOIDY (ESA) 3-CHROMOSOME FISH ASSAY.

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The murine epididymal sperm aneuploidy (ESA) 3-chromosome FISH assay, previously developed in our laboratory, utilizes DNA probes specific for mouse chromosomes X, Y and 8. The objective of this study was to assess the reliability and reproducibility of this assay by comparing the frequencies of aneuploid sperm by scorer, probe-labeling procedure and laboratory. For the inter-scorer comparison, a total of 130,787 cells were analyzed by 2 scorers (5000 cells/mouse/scorer) on the same 13 mice. The frequencies of aneuploid sperm [XX8, YY8, XY8, 88(X or Y) and diploidy] were not significantly different ($p>0.05$) when the two scorers were compared. Recently, fluorochrome-incorporated probes were introduced to the ESA assay which provides brighter, more compact signals and less background than the indirect probes, but it also eliminates the extensive washing and staining procedures. Two different methods of probe-labeling (direct vs. indirect) were evaluated on the same five mice (10,000 sperm/method/animal). The frequencies for hyperhaploidy of the sex chromosomes and chromosome 8 were not significantly different ($p>0.05$). Lastly, the frequencies of aneuploid sperm from 3 mice (10000 cells/animal) were evaluated by two different laboratories (Adler et al. 1996) using similar procedures. There were no significant differences in most categories ($p>0.05$). It can be concluded that data generated using the ESA FISH assay are reliable across scorers, probe-labeling procedures, and that the assay can be replicated in other laboratories. [Work was performed under the auspices of the U.S. DOE by the Lawrence Livermore National Lab. under contract W-7405-ENG-48; with support from NIEHS Y01-ES-10203-00].